Polarographic Behavior of 12-Ketosapogenins

By Constantine Ricciuti, C. O. Willits, M. E. Wall and M. M. Krider

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Steroidal compounds containing α,β-unsaturated keto groups are polarographically reducible. Eisenbrand and Picher[1] and Sartori and Bianchi[2] found that steroids such as testosterone, progesterone, pregnenol-17-one-3 and desoxycorticosterone, are reducible at the dropping electrode in aqueous ethanol solutions and give waves which are proportional to concentration. The polarographic method was applicable only to the Δ^4-unsaturated-3-ketosteroids, for their saturated analogs did not give polarographic waves. Wolfe, Hershberg and Fieser[3,4] investigated Δ^1-cholestenone and found that it was reducible. All of these reducible compounds contain an α,β-unsaturated keto group. They also extended the polarographic method to include 17-ketosteroids and 20-ketosteroids by reaction of these steroids with Girard Reagent T to form polarographically reducible Girard derivatives.

There have been no previous reports on the polarographic behavior of 12-ketosapogenins which are important as precursors in steroid syntheses. The present method for the determination of polarographic behavior of these compounds allows the use of a non-aqueous medium consisting of a lithium chloride methanol–benzene electrolytic solution in which the 12-ketosapogenins are soluble.

Experimental

A Sargent Model XXI Polarograph was used to obtain the current–voltage curves. The capillary had t and m values of 1.35 seconds and 3.587 mg. per sec., respectively, which gave a capillary constant of 2.46 mg.$^{2/3}$sec.$^{-1/2}$. The m and t values were obtained in an open circuit, with the polarographic cell maintained at 25.0°, and with the capillary dipping into the non-aqueous electrolytic solution. The capillary constant at −1.80 volts under the above conditions was 2.38. This value has been used to calculate the diffusion current constant of the 9,11-dehydromanogenin.

The electrolytic cell was a modified Lingane H-cell[5] with a saturated calomel reference electrode. This cell had an open circuit resistance of 1175 ohms, and all half-wave potentials were corrected for IR drop. Half-wave potential readings were made against the saturated calomel electrode, and the polarograms were obtained at 25 ± 0.1°. The sapogenins were isolated and characterized by methods developed at this Laboratory.[6] Descriptive data for these compounds are presented in Table I. Thirty milliliters of the electrolytic solution, consisting of 0 3 M lithium chloride in a 50–50 (by volume) mixture of absolute methanol–benzene, was measured into the sample arm of the H-cell. The solution was degassed with high-purity nitrogen, and a polarogram was recorded. The sample was then added (10–40 mg.), and the solution was again degassed with nitrogen. A polarogram was recorded, and from the increase in wave height, the diffusion current of the reducible sapogenin was calculated.

Table II

Polarographic Characteristics of 9,11-Dehydromanogenin

Wt. sample per 40 ml.	Mole per liter	i_d, μa.	i_d/C	$i_d/Cm^{2/3}t^{1/6}$
0.0121	0.000885	4.76	5516	2.32
.0258	.001887	10.10	5489	2.31
.0390	.003019	15.70	5643	2.37

Results and Discussion

Tigogenin, which has no keto group, shows no polarographic reduction. Hecogenin and manogenin, both having a 12-keto group but no unsaturated linkage, show no reduction. Kammogenin, which has a 12-keto group and a Δ^5-unsaturated linkage, also does not reduce. However, 9,11-dehydromanogenin, which has both a 12-keto group and a conjugated unsaturated linkage, reduces at the dropping electrode with a half-wave potential of −1.72 volts *vs.* S.C.E. and has a diffusion current constant of 2.33. This diffusion current constant is similar to that found for other conjugated ketones in the non-aqueous electrolyte. Mesityl oxide, for example, has a diffusion current constant of 2.07. The reducibility of the 12-keto group of 9,11-dehydromanogenin in the non-aqueous electrolyte was expected because of the conjugated carbonyl group in this compound. Table II shows that the wave height of the 9,11-dehydromanogenin is directly proportional to concentration in the range studied (10 to 40 mg./30 ml.). The use of the lithium chloride non-aqueous electrolyte made possible the direct polarographic analysis of these water-insoluble steroidal compounds.

Table I

Identification Characteristics of Sapogenins Used in This Study

Sapogenin	Melting point, °C.[a] Genin	Acetate	Specific rotation[b] Genin	Acetate	Infrared absorption
Tigogenin	207–209	205–206	−70	−74	Carbonyl absent
Hecogenin	260–261	245–246	+ 7	− 5	Carbonyl max. at 1706 cm.$^{-1}$
Kammogenin	241–243	253–254	−54	−80	Carbonyl max. at 1714 cm.$^{-1}$
Manogenin	244–246	248–250	− 2	−42	Carbonyl max. at 1709 cm.$^{-1}$
9,11-Dehydromanogenin[c]	232–233	258–260	− 7.8	..	Carbonyl max. at 1676 cm.$^{-1}$ C=C max. at 1602 cm.$^{-1}$

[a] All melting point determinations made with the Kofler block. [b] Rotations determined at 25°, sodium lamp, concentrations between 8–10 mg./ml. [c] 95% pure, as estimated from ultraviolet and infrared absorption, with approximately 5% of a non-conjugated carbonyl sapogenin.

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Eastern Regional Research Laboratory[7]
Philadelphia 18, Pennsylvania

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(7) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.